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Associations between oral HPV16 infection and cytopathology: evaluation of an oropharyngeal “Pap-test equivalent” in high-risk populations

Carole Fakhry, MD, MPH¹, Barbara T. Rosenthal, BA, CT², Douglas P. Clark, MD², and Maura L. Gillison, MD, PhD^{3,*}

¹Johns Hopkins Medicine Department of Otolaryngology-Head and Neck Surgery

²Johns Hopkins Medicine Department of Pathology

³Ohio State University, James Comprehensive Cancer Center

Abstract

Human papillomavirus (HPV) is responsible for the rising incidence of oropharyngeal squamous cell cancers (OSCC) in the United States (U.S.), and yet, no screening strategies have been evaluated. Secondary prevention by means of HPV detection and cervical cytology has led to a decline in cervical cancer incidence in the U.S. Here, we explored an analogous strategy by evaluating associations between HPV16 infection, cytopathology and histopathology in two populations at elevated risk for OSCC. In the first, a cross-sectional study population (PAP1), cytology specimens were collected by means of brush biopsy from patients presenting with oropharyngeal abnormalities. In the second (PAP2), a nested case-control study, bilateral tonsillar cytology samples were collected at 12-month intervals from HIV-infected individuals. The presence of cytopathological abnormality in HPV16-positive tonsil brush biopsies (cases) was compared to HPV16-negative samples (controls) matched on age and gender. HPV16 was detected in samples by consensus primer PCR and/or type-specific PCR. Univariate logistic regression was used to evaluate associations. In PAP1, HPV16 alone (OR 6.1, 95% CI 1.6–22.7) or in combination with abnormal cytology (OR 20, 95% CI 4.2–95.4) was associated with OSCC. In PAP2, 4.7% (72 of 1524) of tonsillar cytology specimens from HIV-infected individuals without oropharyngeal abnormalities were HPV16-positive. Tonsillar HPV16 infection was not associated with atypical squamous cells of unknown significance (ASCUS), the only cytological abnormality identified. Therefore, HPV16 was associated with OSCC among individuals with accessible oropharyngeal lesions, but not with cytological evidence of dysplasia among high-risk individuals without such lesions. An oropharyngeal Pap-test equivalent may not be feasible, likely due to limitations in sampling the relevant tonsillar crypt epithelium.

Keywords

oral HPV; tonsillar HPV; head and neck squamous cell cancer; cervical HPV; head and neck cancer screening

BACKGROUND

Human papillomavirus (HPV) is etiologically responsible for a distinct and growing subset of oropharyngeal squamous cell cancers (OSCC) that disproportionately affect young men,

*Corresponding author: 690 Tzagournis Research Facility, 420 West 12th Avenue, Columbus, OH 43221.

nonsmokers and nondrinkers (1, 2). In contrast to OSCC, cervical cancer incidence rates have declined for decades in the United States (U.S.) because of a successful secondary prevention strategy by means of cervical cytology screening (Papanicolaou or “Pap” test) (3–5). Because of its superior sensitivity, cervical HPV detection has been incorporated into the screening algorithm and may replace cervical cytology (6–8). Given this, it is reasonable to investigate whether HPV detection and/or an oral “Pap test equivalent” could be similarly used to screen for HPV-positive OSCC.

HPV16 is estimated to confer at least a 15-fold increase in risk for OSCC in case-control studies (1). In cervical cancer screening, the presence of a high-risk HPV infection is associated with an approximate 50-fold increase in risk of cervical cytological abnormalities (e.g. cervical intraepithelial neoplasia, CIN 1-3) when compared with HPV-negative women (9). We therefore investigated whether or not oral HPV16 infection is associated with cytological or histological abnormalities of the oropharynx in two populations: 1) patients who present for evaluation of clinical abnormalities of the oropharynx (PAP1) and 2) a population known to have a two to sixfold increase in risk for both oral HPV infection and OSCC, HIV-infected individuals (PAP2) (10–12).

METHODS

Study populations and specimen collection

Cross-sectional study population (PAP1)—Eligible subjects included patients with oropharyngeal abnormalities referred to a head and neck surgeon for diagnostic evaluation. Patients were enrolled at Johns Hopkins Hospital and affiliated centers (Greater Baltimore Medical Center), University of Maryland, University of Pittsburgh and Louisiana State University. Patient demographic characteristics, risk behaviors (alcohol and tobacco use) and clinical characteristics of the lesion (including color, appearance, ulceration, symptoms, location, duration, size and clinical impression) were obtained by a standardized, clinician-completed questionnaire. Cytology specimens were obtained from all lesions by use of an Oral CDx brush (Oral CDx laboratories, Suffern, NY) as recommended by the manufacturer(13). After transfer of cellular material to a glass slide, the cytobrush was placed into a vial containing one milliliter (mL) of Digene Specimen Transport Medium (STM) (Qiagen, Valencia, CA) and stored at -80°C until further processing (see below). Incisional biopsy was performed for all but one lesion which was benign appearing.

Nested case-control (PAP2)—A case-control study was nested within a prospective cohort study of the natural history of oral HPV infection in HIV-positive men and women (HOPE) at The Johns Hopkins Hospital, Baltimore, MD. Oral samples were obtained for HPV detection every six months for a maximum of six visits.

Oral rinses were collected at six-month intervals by use of a 30-second oral rinse and gargle with 10-mL of Scope mouthwash. Oral exfoliated cells were centrifuged, washed with phosphate buffered saline (PBS), and stored at -80°C until further processing.

Bilateral tonsillar brush biopsies were performed at 12-month intervals by use of two Oral CDx brushes. Circumferential pressure was applied along the palatine tonsils or posterior pharyngeal wall (in patients without tonsils) until pinpoint bleeding was elicited. The cytobrush was placed in a vial containing 1mL of PBS on ice for transport to the laboratory. The samples were vortexed, split into two equal aliquots and centrifuged. One aliquot was re-suspended in 0.5 mL Digene STM (Qiagen, Valencia, CA) and the second in 0.5 mL of Cytosch Red (BD, Franklin Lakes, NJ). Pilot studies were performed to determine the optimal specimen fixative for preservation of cytoarchitecture with freeze-thaw. Cytosch Red was found to best preserve cytological architecture (Figure 1 is representative of

cytology samples preserved in Cytorich Red). Samples were stored at -80°C until further processing.

Specimen processing and HPV detection—DNA was purified from oral rinse samples by use of a modified protocol for a Puregene DNA Purification Kit (Gentra Systems, Minneapolis, MN) (14). DNA was purified from cytology specimens (both oropharyngeal cytology specimens and tonsillar cytology specimens) in Digene STM by standard protocol. Briefly, half of the STM sample was added to a proteinase K-digestion buffer solution (final concentration 0.4 mg/ml proteinase K, 50 mM Tris-HCl, 1 mM EDTA, 0.5% Tween-20) and incubated at 65°C for one hour followed by heat inactivation at 95°C for 10 min. DNA was precipitated by standard ammonium acetate ethanol precipitation, washed with 70% ethanol, dried and re-suspended in 20 μl of LoTE buffer (3mM Tris-HCl and 0.2 mM EDTA).

The presence of HPV16 genomic DNA was detected in oropharyngeal cytology and oral rinse samples by PCR amplification by use of PGMY09/11 L1 consensus primers, followed by hybridization to a prototype HPV probe linear array and β -globin (Roche Molecular Systems, Inc., Alameda, California)(15). A negative control was included for each row of the 96-well plate (1 in 11 experimental samples). Samples that were β -globin negative were considered to be of insufficient quality for analysis.

Type-specific, quantitative, TaqMan real-time PCR (Applied Biosystems, Foster City, CA) targeted to HPV16 E6 was used to detect HPV16 in oropharyngeal (PAP1) and tonsillar cytology samples (PAP2) (16). A negative control was included for each row of the 96-well plate (1 in 10 experimental samples). Samples with a mean of the duplicates greater than or equal to one viral copy were considered positive.

For oropharyngeal and tonsillar cytology samples, the number of epithelial cells in each PCR reaction was estimated by use of a TaqMan real-time PCR targeted to a single copy human gene on chromosome 7, Human Endogenous Retrovirus 3 (ERV-3) (14). Data were used to: (1) categorize PAP2 tonsillar cytology samples as evaluable if ERV3 was greater than or equal to three (lower limit of assay sensitivity); (2) to adjust HPV16 viral load to cell number and; (3) to estimate the number of cells evaluated by cytopathology.

All specimen processing and analysis was performed in the Gillison laboratory (Johns Hopkins Medicine, Baltimore, Maryland).

Cytology PAP1—Cytology slides from patients with oropharyngeal abnormalities were interpreted at OralCDx Labs by pathologist-guided computer analysis and reported as “not interpretable” or “interpretable”. Samples considered “not interpretable” were incomplete transepithelial biopsies that were not representative of all three epithelial layers (13). “Interpretable” slides were further categorized as “negative”, “atypical” or “positive”.

Cytology PAP2—To evaluate associations between tonsillar HPV16 infection and cytological abnormality in PAP2, a nested case-control study was performed. A case was defined as an HPV16-positive tonsillar cytology specimen. A control was defined as an HPV16-negative tonsillar cytology specimen from an individual with a corresponding HPV16-negative oral rinse specimen. Controls were matched to cases in a two-to-one ratio on subject study visit, gender and age (in five-year categories). Tonsillar cytology specimens from both cases and controls underwent cytopathological review. Additionally, cytopathological analysis was performed on contralateral HPV16-negative tonsillar cytology specimens from cases as well as specimens from all individuals with an HPV16-positive oral rinse sample in the presence of HPV16-negative tonsillar cytology specimens.

Tonsillar cytology specimens in Cytorich Red from the nested case-control study were prepared by thawing to room temperature and applied to slides using cytocentrifugation. Briefly, approximately 300 μL of each specimen was combined with 300 μL of Shandon Cytospin Collection Fluid (Thermo Fisher Scientific, Kalamazoo, MI) and applied to two cytospin slides (6 mm area each) using a cytospin funnel and centrifugation. Slides were then stained using routine Papanicolaou stain.

A case-report form for interpretation of the tonsillar cytology specimens was created to standardize cytological evaluation and reporting and was derived from the Bethesda System criteria used for evaluating squamous lesions in cervical cytology (17). Specimens were evaluated for adequacy based on the presence of at least 1000 squamous cells and lack of obscuring factors such as blood or inflammation. Slides were assigned to one of the following categories: 1) Negative for dysplasia or malignancy; 2) positive for atypical squamous cells of undetermined significance (ASCUS); 3) Positive for dysplasia (mild, moderate, or severe dysplasia); 4) Positive for squamous cell carcinoma. All slides were screened by an experienced cytotechnologist (BTR) and interpreted by an experienced cytopathologist (DPC). Both reviewers were masked to HPV status and clinical features of individual cases during evaluation.

In situ hybridization (ISH)—Available tumors were evaluated for the presence of HPV16 DNA by use of the in situ hybridization–catalyzed signal amplification method for biotinylated probes (GenPoint; Dako, Carpinteria, CA), as previously described (18). Slides were scored as positive for HPV16 if a punctate signal specific to tumor cell nuclei was present.

Statistical analysis

Characteristics of each study population were summarized using descriptive statistics. PAPI cytology and histopathology were categorized as dichotomous variables of normal or abnormal (including “atypical” or “positive”) and SCC or non-SCC, respectively. ISH scoring was categorized as a dichotomous variable of negative and positive. In PAP2, HPV16 infection status was categorized by sample (oral rinse or tonsillar cytology), study visit (1 to 6) and by study subject (N=401). Detection of HPV16 infection was described as visit-specific or cumulative prevalence. A subject was considered infected if HPV16 was present at each visit (prevalence) or detected in any samples collected over the course of the study (cumulative prevalence).

Associations between HPV16 and abnormal cytology or histology were evaluated using univariate logistic regression (odds ratios and 95% confidence intervals [95%CI]). A two sided p-value less than 0.05 was considered statistically significant. Stata IC 11 software (College Station, Texas) was used to perform the analysis.

RESULTS

PAP1

A total of 92 individuals with clinically apparent lesions of the oropharynx were enrolled in PAPI. The majority was male (78.3%) and Caucasian (85.9%). Median age was 56.5 years (range 22–85). The majority had a history of tobacco smoking (65.2%), and current smokers had a median 40.5 (IQR 30, 60) pack-years of use. Twenty-five percent (23 of 92) reported heavy current alcohol use (>5 drinks per day). The anatomic site distribution of the oropharyngeal abnormality that underwent cytological analysis was tonsil (n= 36, 39.1%), base of tongue (34, 37.0%), posterior pharyngeal wall (6, 6.5%), retromolar trigone (4, 4.4%), and mixed/other (8, 8.6%). Cytology specimens were collected under general

anesthesia (82.6%), in an outpatient clinic (9.8%) or location unknown (7.6%). The clinical characteristics of the oropharyngeal abnormalities in PAP1 are summarized in the Supplementary Table.

All but one of the oropharyngeal cytology specimens were s-globin positive and therefore evaluable for HPV. HPV DNA was detected by Roche linear array in 52.8% (48 of 91) of evaluable samples. The HPV type-specific distribution for positive samples was HPV16 (n=41, 85.4%), HPV33 (4, 8.3%) and HPV11, 18, 35, 52, 67, 72, 81 and 83 (for each, n=1, 2.1%). Five samples were co-infected with two distinct HPV infections. HPV16 was detected by type-specific quantitative PCR in 97.6% (40 of 41) of samples positive by linear array and two additional samples were positive. The median HPV16 viral load among the 43 positive samples was 1.49 copies per cell (95%CI 0.25–6.52). As measured by ERV3, a median of 17,267 cells (IQR: 3934, 60933) were evaluated per sample by real-time PCR for presence of HPV16 in oropharyngeal cytology specimens.

In cytopathological analysis, 84.8% (78 of 92) of oropharyngeal cytology specimens were interpretable, and of these 78 samples, 14 (17.9%) were normal, 59 (75.6%) were atypical and 5 (6.4%) were positive. Therefore, a total of 64 (82.1%) were considered abnormal in our analysis. Detection of HPV16 by type-specific PCR was associated with a non-significant three-fold increased in odds of abnormal cytology (OR 3.2, 95%CI 0.9–11.3, Table 1). Furthermore, the presence of high-risk HPV by linear array (OR 4.6, 95% CI 1.4–15.4) or HPV16 by real-time PCR (OR 6.1, 95%CI 1.6–22.7, Table 2) was strongly associated with a histopathological diagnosis of squamous cell carcinoma among the 91 lesions for which histopathology was available. Abnormal cytology was also strongly associated with squamous cell carcinoma (OR 8.0, 95%CI 2.2–29.6, table 2). The combination of HPV16 and abnormal cytology was strongly associated with OSCC (OR 20, 95%CI 4.2–95.4).

To explore whether or not the presence of the HPV-negative subset of OSCC could explain the weak association between HPV and abnormal cytology, a stratified analysis was performed. Tumor HPV16 status was available for 67% of biopsy specimens (61 of 91). HPV16 was indeed detectable in a higher proportion of abnormal cytology samples (49 of 61) from study subjects with HPV16-ISH positive than negative tumors (91.3% [21 of 23] vs. 38.5% [10 of 26]). HPV16 presence in cytology samples appeared to be associated with HPV16-positive (OR 23, 95%CI 0.75–702.6), but not HPV16-negative OSCC (OR 0.8, 95%CI 0.6–10.0).

PAP2

Given the findings noted above, a study was designed to evaluate associations between HPV16 infection and tonsillar cytopathology in a population at high-risk for HPV infection and tonsillar cancer, HIV-infected individuals.

A total of 401 HIV-infected men and women were enrolled in the HOPE cohort study. The HOPE cohort was 60.1% (n=241) male and 86.3% (n=346) African-American, and the overwhelming majority were non-Hispanic (n=390, 97.3%). Median age was 46.4 years (range 20.7–73.9). Median CD4 count was 304 cells per μ L (IQR 176, 602) and median HIV viral load was 354.0 copies per mL (IQR 55.5, 26470.0). There were no discernable clinical abnormalities of the oropharynx.

A total of 1,198 oral rinse samples were collected at visits 1–5 and 99.1% (1187 of 1198) were evaluable for HPV16 by linear array. The prevalence of oral HPV16 infection among 1,187 evaluable oral rinses collected was 2.8% (95%CI 1.8–3.7). The prevalence of oral

HPV16 infection in the study population was 2.6%, 1.3%, 2.8%, 2.7% and 5.9% at visits 1–5, respectively.

Bilateral tonsillar brush biopsy samples were collected at the baseline visit from all 401 enrolled subjects and from 247 and 136 subjects at the 12- and 24- month visits, respectively. Thus, a total of 1,551 tonsillar cytology samples were collected (17 individuals refused to undergo collection of second tonsillar brush biopsy at one of the visits) and 1,524 (98.3%) were evaluable as determined by ERV3. Overall, HPV16 was detected by quantitative PCR in 4.7% (95%CI 3.7–5.8%). Median HPV16 viral copy number among positive samples was 2 per 1000 ERV3 copies (IQR 0.3,39.3).

The cumulative prevalence of individuals with tonsillar HPV16 infection at last visit was 11.8% (47 of 397). Tonsillar cytology specimens were HPV16-positive for 6.9% (27 of 394) at visit one, 9.1% (22 of 242) at visit three and 6.0% (8 of 133) at visit five (Figure 2A). Among the 47 individuals who had tonsillar HPV16 infection, 10 had concurrent bilateral tonsillar infections. Patterns of HPV16 infections among all individuals with a detectable tonsillar infection are shown in Figure 2A. In total, 72 HPV16-positive tonsillar cytology samples were collected from 47 individuals, and these comprised the case population. The majority of oral HPV16 infections (76%, 19 of 25) identified at visits 1, 3, and 5 were found in the presence of a tonsillar HPV16 infection (Figure 2B) and conversely only 34.5% (19 of 55 evaluable paired oral rinse samples) of tonsillar infections were found in the presence of an oral HPV infection.

To evaluate the association between tonsillar HPV16 infection and cytopathology, all 72 HPV16-positive tonsillar cytology samples and 134 matched HPV16-negative cytology specimens underwent cytopathological review. Samples from two cases and eight controls were categorized as unevaluable by the pathologist. As estimated by ERV3, a median of 65,509 (IQR: 29,285, 128,589) tonsillar epithelial cells were evaluated per Pap-test equivalent.

On cytological review, koilocytosis, a marker of nuclear changes consistent with HPV infection, was not detected in tonsillar cytology specimens (99.7%, 284 of 285). ASCUS was present in 8.6% (6 of 70) and 4.8% (6 of 126) of evaluable cases and controls, respectively (Figure 1 is representative of normal and abnormal cytology). Neither dysplasia nor cancer was detected in any of the specimens. The presence of tonsillar HPV16 infection was associated with a nonsignificant increase in odds of ASCUS (OR 1.9, 95%CI 0.48–7.3, table 1). ASCUS was also observed in 11.9% (5 of 42) of evaluable cytology specimens from the contralateral tonsil of individuals with a tonsillar HPV16 infection. All individuals without tonsillar HPV16 infection but with a detectable oral HPV16 infection were without any detectable cytopathology (n=26).

DISCUSSION

In a study population with high prevalence of HPV and clinical oropharyngeal abnormalities (PAP1), the combination of HPV16 and abnormal cytology was strongly associated with OSCC. In contrast, in a study population (PAP2) with high prevalence of oral HPV16 infection (20) and elevated risk for tonsillar SCC (10–12), HIV-infected individuals, tonsillar HPV16 infection was not associated with cytological changes consistent with HPV infection or dysplasia. The only cytopathological abnormality detected in the study population was ASCUS, and its presence was not associated with tonsillar HPV16 infection.

The primary purpose of a screening test is to identify individuals with precursor lesions or early stage cancer more amenable to treatment (e.g. PAP2). Although HPV16 infection and abnormal cytology were strongly associated with OSCC in those with oropharyngeal

abnormalities (PAP1), we could not detect tonsillar dysplasia among individuals with either a tonsillar or oral HPV16 infection (PAP2). These results suggest that an oropharyngeal Pap-test equivalent may not be feasible as a secondary prevention tool for HPV-positive OSCC. However, our study had limitations, and therefore further study may be warranted.

There are several possible explanations for why we may not have observed an association between HPV16 and tonsillar cytopathology. Although HIV-infected individuals have a two- to six- fold increased risk for tonsillar SCC (10, 11, 21, 22), tonsillar cancer is a rare disease, and therefore our study population may not have provided sufficient power to detect an association. Despite the high cumulative prevalence (11%) of tonsillar HPV16 infection, we observed no cellular changes consistent with dysplasia. In studies of comparable sample size, strong associations between high-risk cervical HPV infections and dysplasia have been observed (23).

We acknowledge that we may have misclassified individuals as high-risk HPV-negative by focusing exclusively upon HPV16. However, approximately 95% of HPV-positive tonsillar cancers are HPV16-positive (24, 25) and no dysplasia was observed in HPV16-negative samples. This is therefore unlikely to explain our null associations. By analogy to cervical disease, persistent high-risk HPV infection would be expected to be a reasonable surrogate for those at risk for tonsillar dysplasia (26). Although our study design allowed us to identify some individuals with a persistent tonsillar HPV16 infection, the majority of infections were transient. However, in cervical cancer, even transient infections are associated with a 5-fold increased risk of squamous intraepithelial lesion (27).

Laboratory contamination is unlikely to explain our results because all negative controls were appropriately negative. The high agreement we observed between HPV16 detection in tonsillar cytology and oral rinse samples collected at the same visit argues against contamination, as samples were independently processed and evaluated. This high agreement might reflect “contamination” of tonsillar samples by HPV16 infection at other sites within the oral cavity. However, we have previously found HPV16 detected in oral rinse samples to be identical in sequence to the HPV16 detected in individuals with tonsillar cancer (28).

We believe that the most likely explanation for our results is related to an inability to sample the relevant tonsillar epithelium. We observed associations between HPV16 and squamous cell carcinoma in oropharyngeal lesions that could be sampled with direct visualization. Despite the majority of oropharyngeal and tonsillar cytology specimens being evaluable with adequate cellularity, the squamous epithelium of significance may have been inaccessible with the oropharyngeal Pap-test equivalent in PAP2. HPV-positive tonsillar cancers are believed to arise from deep within the involuted tonsillar crypt epithelium (29), rendering a superficial brush biopsy insufficient for sampling the relevant cells. This is analogous to the known limitation of cervical Pap tests for screening of cervical adenocarcinomas (30). Cervical Pap tests efficiently sample the superficial squamous epithelium of the ectocervix, however they do not thoroughly sample the glandular cells of the endocervix. In support of this is the significant increased incidence of cervical adenocarcinoma in the U.S. while squamous cell carcinoma incidence has declined in the same time period (31).

Despite our study limitations, this study represents the first attempt to evaluate an oropharyngeal Pap-test equivalent for HPV-positive cancers at this anatomic site. Our data indicate that any screening modality for OSCC will have to allow both visualization and sampling of lesions deep within the tonsillar crypts. We envision using such a screening

modality in a population at increased risk for development of OSCC such as individuals with persistent oral HPV16 infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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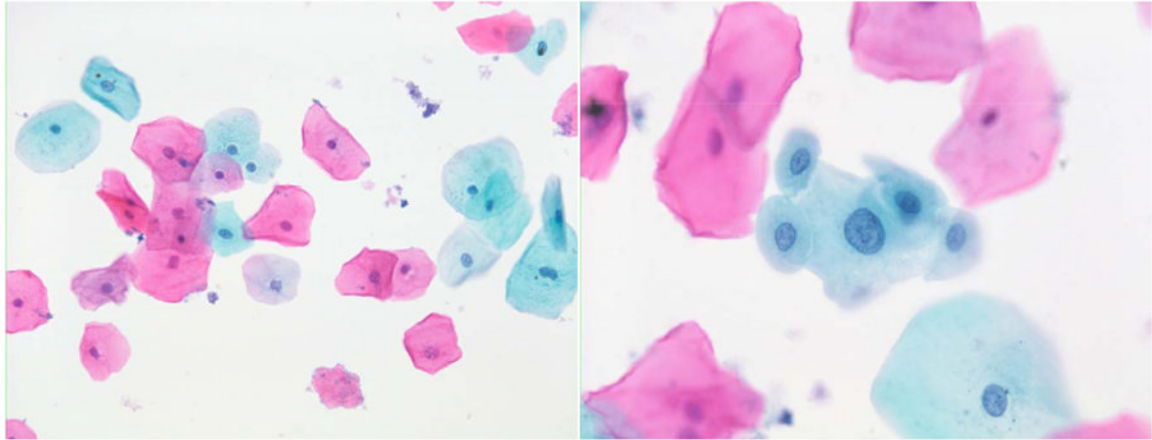


Figure 1. Cytological appearance of normal-appearing superficial squamous cells (left panel; magnification x 200, Papanicolaou stain) and ASCUS of a tonsillar cytology sample (right panel; magnification x 400, Papanicolaou stain). Note the cell in the center with slight nuclear enlargement and mild nuclear hyperchromasia relative to the surrounding normal squamous cells.

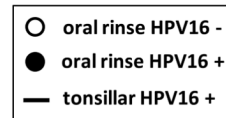
Figure 2a.

ID Number	1 visit (baseline)	3 visit (12-months)	5 visit (24-months)
2	●	●	●
4	●	●	●
7	●	●	○
27	●	○	○
33	○	○	●
34	○	●	○
63	●	○	○
65	○	●	○
67	●	●	●
71	○	●	○
82	●	○	○
91	○	●	○
97	○	●	○
102	○	○	●
115	○	●	●
118	○	○	●
144	○	●	○
157	○	●	○
170	○	●	○
172	○	●	●
188	○	●	○
209	○	●	○
221	○	●	○

● HPV16 +
○ HPV16 -

Figure 2b.

ID Number	1 visit (baseline)	2 visit (6-months)	3 visit (12-months)	4 visit (18-months)	5 visit (24-months)
2	●	●	●	●	●
4	○	○	●	○	●
7	○	○	●	●	○
35	○	○	○		●
50	○	○	○	○	●
67	●	●	●	●	●
91	○	○	○	○	●
115	●		●		●
172	○	○	●	○	●
62	●	●	●	●	
113	○	○	○	●	○
197	●		○		
283	●	○	○	○	
309	●		○	○	
6	●	○			
292	●				
366	●				

**Figure 2.**

HPV16 infection status in HIV-infected individuals over time. Oral rinses were collected from subjects at visits 1 (baseline), 2 (6-months), 3 (12-months), 4 (18-months) and 5 (24-months). Tonsillar samples were collected at visits 1, 3 and 5. Oral rinse samples and tonsillar cytology samples were evaluated for the presence of HPV16. Figure 2A shows individuals who were present for all three visits and had at least one detectable tonsillar HPV16 infection. Shaded circles are tonsillar samples which are HPV16-positive. Unshaded circles are HPV16-negative tonsillar samples. In figure 2B, individuals who had an oral HPV16 infection detected during at least one visit are depicted. Shaded circles are oral rinse samples which are HPV16-positive. Open circles indicate HPV16-negative oral rinse samples. Horizontal bar indicates a concomitant tonsillar HPV16 infection. The absence of circle signifies that an individuals did not provide oral rinse sample, was lost to follow-up or sample was unevaluable at a specific visit.

Table 1

Associations between HPV16* and cytopathology

	Cytology normal	Cytology abnormal	Odds ratio
PAP1			(95% CI, p-value)
HPV16 by linear array			
negative	10	30	ref**
positive	4	33	2.8 (0.8–9.7, p=0.12)
HPV16 by real-time PCR			
negative	10	28	ref**
positive	4	36	3.2 (0.9–11.3, p=0.07)
PAP2			
HPV16			
negative	120	6	ref
positive	64	6	1.9 (0.48–7.3, p=0.29)

* HPV16 infection status determined by linear array and RT-PCR in PAP1 and linear array in PAP2. There was a differing number of evaluable samples depending on HPV16

** denotes referent category

Table 2

Association between HPV and diagnosis of SCC in PAPI *

	Non-SCC	SCC	OR
HPV-HR types**			
negative	14	31	ref***
positive	4	41	4.6 (1.4–15.4, p=0.01)
HPV16 real-time-PCR			
negative	15	33	ref
positive	3	40	6.1 (1.6–22.7, p=0.008)
Cytopathology			
negative	7	7	ref
positive	7	56	8.0 (2.2–29.6, p=0.002)

* The total number of cases included in analysis for HR-HPV, HPV16 real-time-PCR and cytopathology differ due to evaluable samples for each respective method and available histopathology.

** denotes high-risk HPV types associated with cervical malignancy

*** referent